

**SEATTLE CHILDREN'S RESEARCH INSTITUTE
OPERATING POLICIES / PROCEDURES**

DEPARTMENT: Institutional Animal Care and Use
Committee
POLICY NUMBER: IACUC-028
REPLACES:
EFFECTIVE DATE: June 10, 2005
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POSTED FROM:

TITLE: Criteria For Review Of Protocols Involving Antibody Production

SUMMARY:

Depending on research needs, antibodies may be produced by either polyclonal or monoclonal technique. Each technique requires that specific issues be addressed in animal protocols.

POLICY/PROCEDURE:

028-1 Adjuvants

028-1.1 Choice of an appropriate adjuvant is important from both the aspect of the end result (high antibody response), and the welfare of the immunized animal. Many adjuvants have the capacity to produce inflammation, tissue necrosis, and pain in animals.

028-1.2 Complete Freund's Adjuvant (CFA) – multiple exposures to this particular adjuvant can cause severe hypersensitivity reactions. The use of CFA can be painful so alternative adjuvants should be considered. Undesirable and painful side effects of this adjuvant can be minimized or avoided by using the following guidelines:

028-1.2.1 Use of appropriate routes of administration and injection volumes not to exceed those listed below for those routes;

028-1.2.1.1 Subcutaneous (SQ), back and flanks: (max. 0.1ml)

028-1.2.1.2 Intramuscular (IM), flank: (max. 0.05ml)

028-1.2.1.3 Intra-dermal (ID): (max. 0.05ml)

028-1.2.1.4 Intraperitoneal (IP), (scientific justification required): (max. 0.1ml)

028-1.2.1.5 **Intravenous (IV) – NOT PERMITTED**

028-1.2.1.6 **Footpad – NOT PERMITTED unless scientifically justified as outlined below**

028-1.2.2 Careful preparation of inoculums and use of aseptic technique;

028-1.2.3 Judicious use of chemical sedation or anesthesia;

028-1.2.4 Adequate separation of injection sites to avoid coalescence of inflammatory lesions, and;

028-1.2.5 Use of Incomplete Freund's Adjuvant (IFA) for subsequent booster immunizations in the same animal.

028-2 Route of Injection

028-2.1 Injection volumes and routes of administration for each agent should be based on manufacturer, and institutional recommendations.

028-2.2 Painful, inflammatory reactions can be minimized by injection of small volumes of inoculum, per injection site, and the use of multiple injection sites.

028-3 Footpad injections:

028-3.1 Requests for use of footpad injection sites, in rodents, require scientific justification that other routes of injection are not feasible for the study. This information will be critically evaluated by the IACUC before approval.

028-3.2 With IACUC approval, footpad injections should involve only one hind foot with animals housed on soft, contact bedding.

028-3.3 Appropriate injection volume guidelines must be followed (0.01ml - 0.05ml in mice; <0.1ml in rats).

028-4 Monoclonal Antibody Production

028-4.1 Protocols for monoclonal antibody (mAb) production should provide sufficient detail regarding methodology for detecting the specific antibody of interest in order to avoid wasting animal resources in later phases of the study.

028-4.2 Ascites method for production of mAb:

028-4.2.1 An *in vitro* method, such as culturing hybridomas in hollow fiber bioreactors or semi-permeable plastic bags, is being developed and offers an alternative to *in vivo* ascites production in some cases;

028-4.2.2 Ascites methods for mAb production should be used only after *in vitro* failure of each cell line has been demonstrated, or other adequate justification is provided;

028-4.2.3 Compliance with the following standards is necessary to minimize discomfort and stress to animals during *in vivo* methods of antibody production:

028-4.2.3.1 Cell lines used for hybridoma formation should be tested for the presence of rodent viruses prior to introduction into the animal colony. Mice inoculated with untested or contaminated cell lines will be quarantined;

028-4.2.3.2 If Pristane is used as a priming agent (for the peritoneal cavity to increase ascites fluid), the dose should not exceed 0.25ml/animal. Other priming agents must be justified and considered by the IACUC on an individual basis;

028-4.2.3.3 After inoculation with an ascites producing hybridoma line, animals must be observed by investigative staff everyday to monitor the degree of abdominal distension and signs of illness;

028-4.2.3.4 Ascites fluid must be proactively aspirated, with the animal under anesthesia, before

abdominal distension causes respiratory distress or interferes with normal activity;

028-4.2.3.5 Animals must be humanely sacrificed, according to the specifications of their approved protocol, when abdominal taps are not successful or the animal appears to be moribund (i.e., as evidenced by lethargy, impaired mobility, or inability to reach food & water), and;

028-4.2.3.6 The number of taps may not exceed a single survival tap and a second terminal tap, unless justified in the protocol and specifically approved by the IACUC.

REFERENCES:

ARENA / OLAW, "The Institutional Animal Care and Use Committee Guidebook", 2nd Ed. NIH. Bethesda, MD. 2002

Submitting Office: Office of Institutional Assurances

Approved by:

\s\ Anne Clancy, IACUC Chairperson, 4/20/2011

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